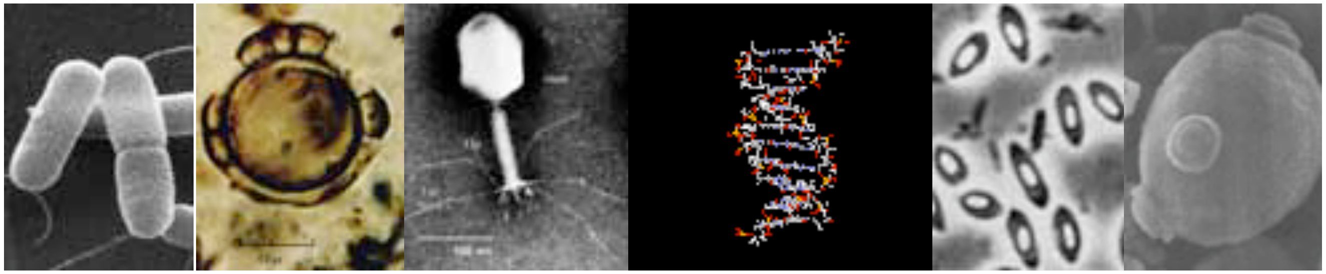


Microscopy and microscopes

General Microbiology - Laboratory

Cañada College - Fall 2008

Instructor: Tamas Torok, Ph.D.

Berkeley Lab

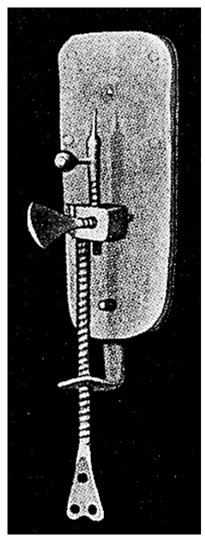
Today’s agenda

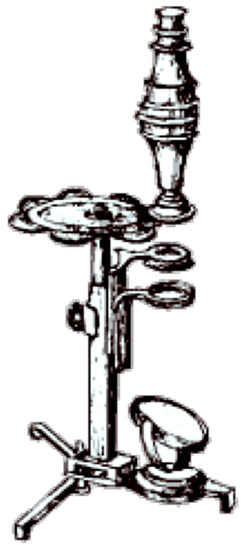
- Bright-field light microscopy
  - history of microscopy
  - today’s light microscopes
  - structure and function of the bright-field light microscope
- Other microscopes

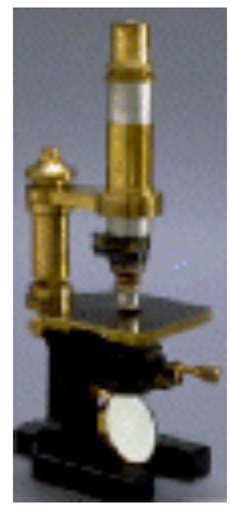
Ernest Abbe (1840-1905)

- "… a non-self-luminous particle, which is illuminated by an extraneous source, gives rise to diffracted light rays…"
- "...to form a good microscopic image as many of the diffracted rays as possible should be intercepted by the objective…"

History of the light microscopy







Leeuwenhoek’s microscope

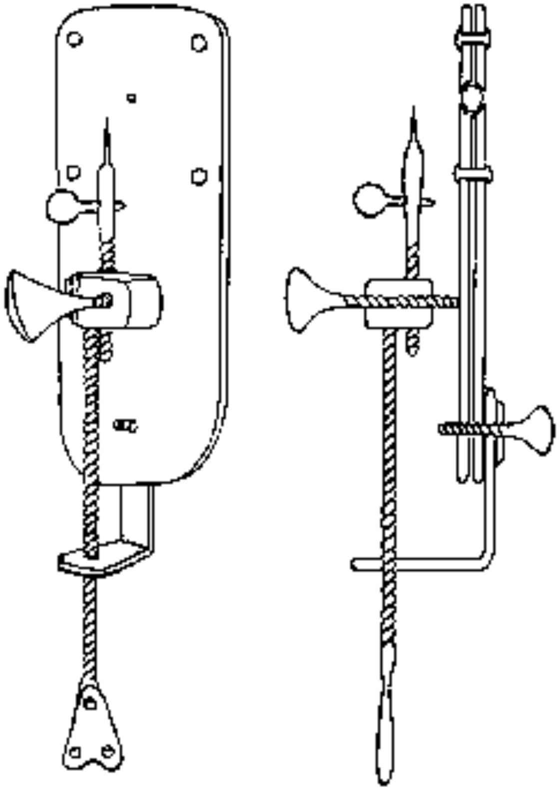
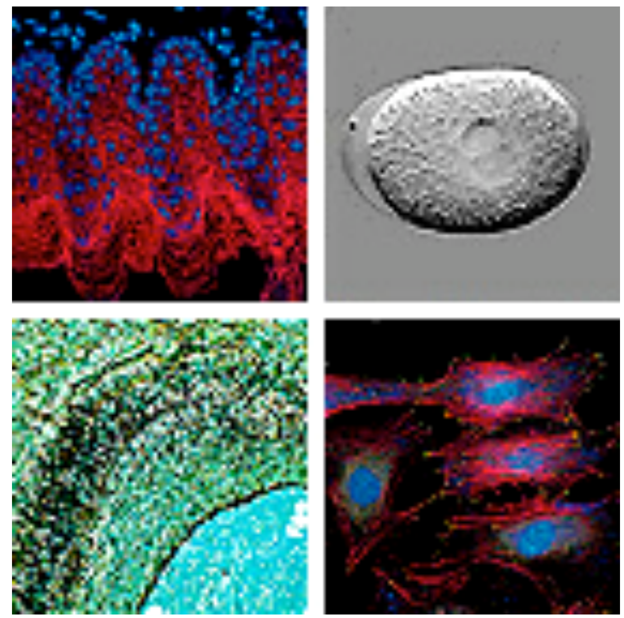


Fig. 1 - Microscope made by Anton van Leeuwenhoek in VIIth century.

State-of-the-art



Abbe's formula

- Resolving power (d)
- Wavelength of light (λ)

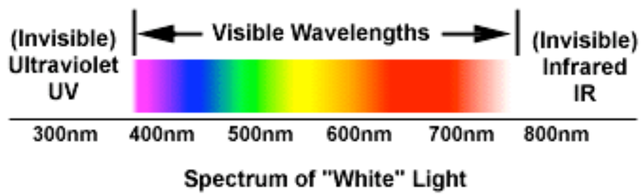


Figure 2

- Index of refraction (n)
- One half the angular aperture (μ)
- Numerical Aperture
- $NA = n (\sin \mu)$
- $d = \lambda/NA = \lambda/n (\sin \mu)$

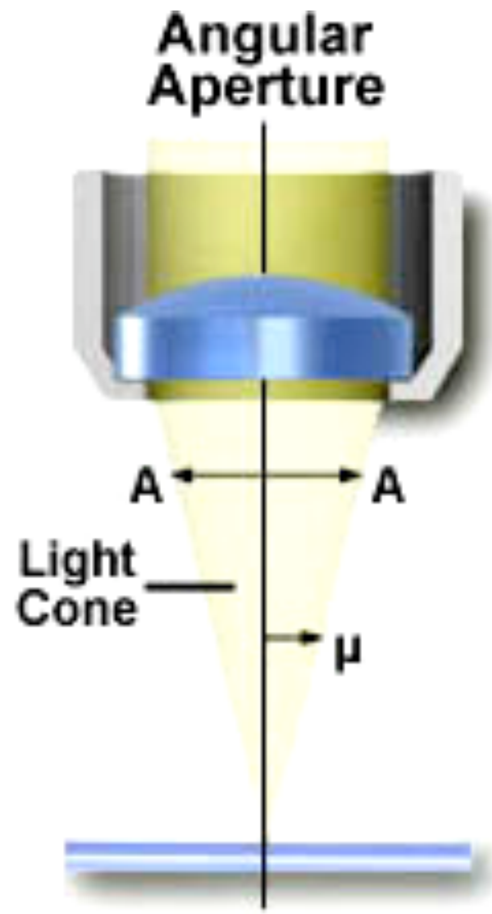
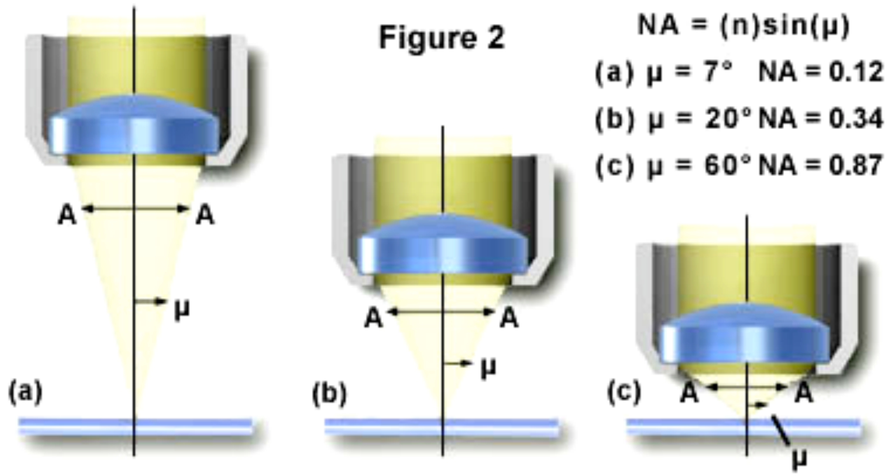


Figure 1

Numerical apperture and resolution

- In confocal and fluorescence microscopy, the resolution may exceed these limits
- Other factors, such as low specimen contrast and improper illumination may lower resolution



Objective information

60x Plan Apochromat Objective

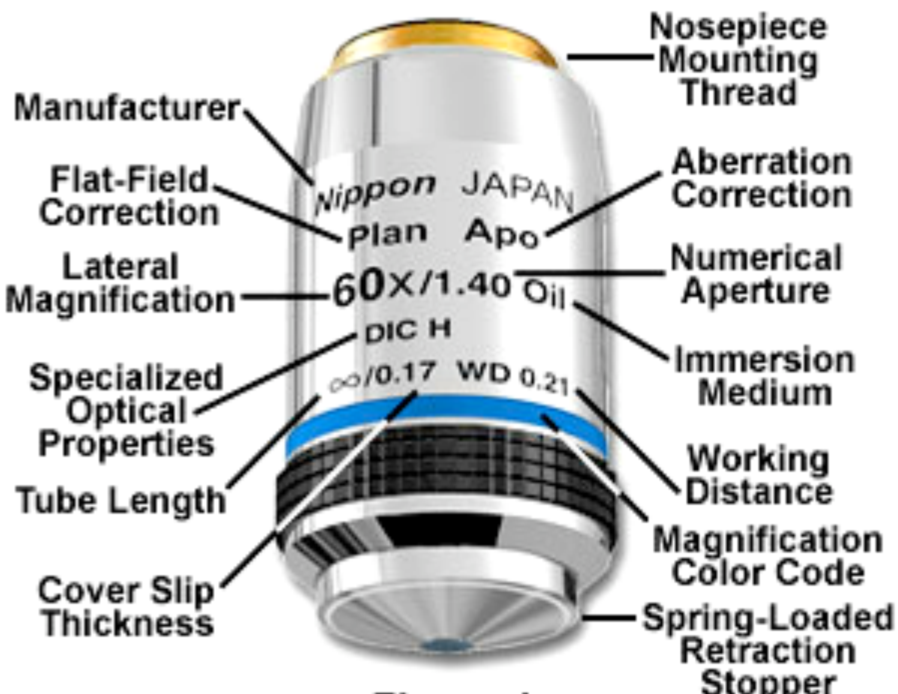


Figure 1

	NA	WD [mm]
10x	0.45	4.00
40x	0.95	0.14
100x oil	1.40	0.13

NA	.25	.50	.95
Depth [µm]	8.0	2.0	.10

Enhancing resolution power

Oil Immersion and Numerical Aperture

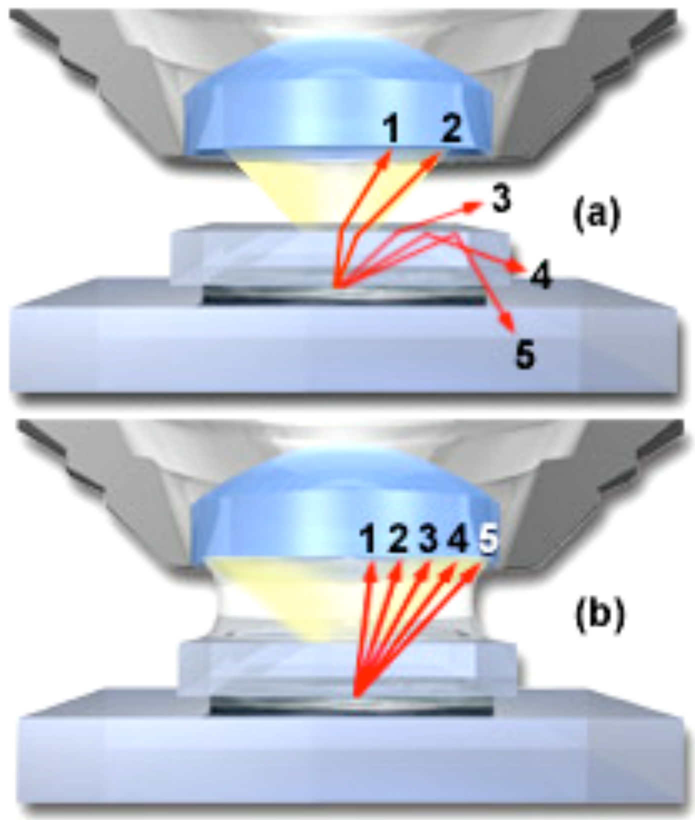


Figure 1

- Numerical aperture is related to the imaging medium

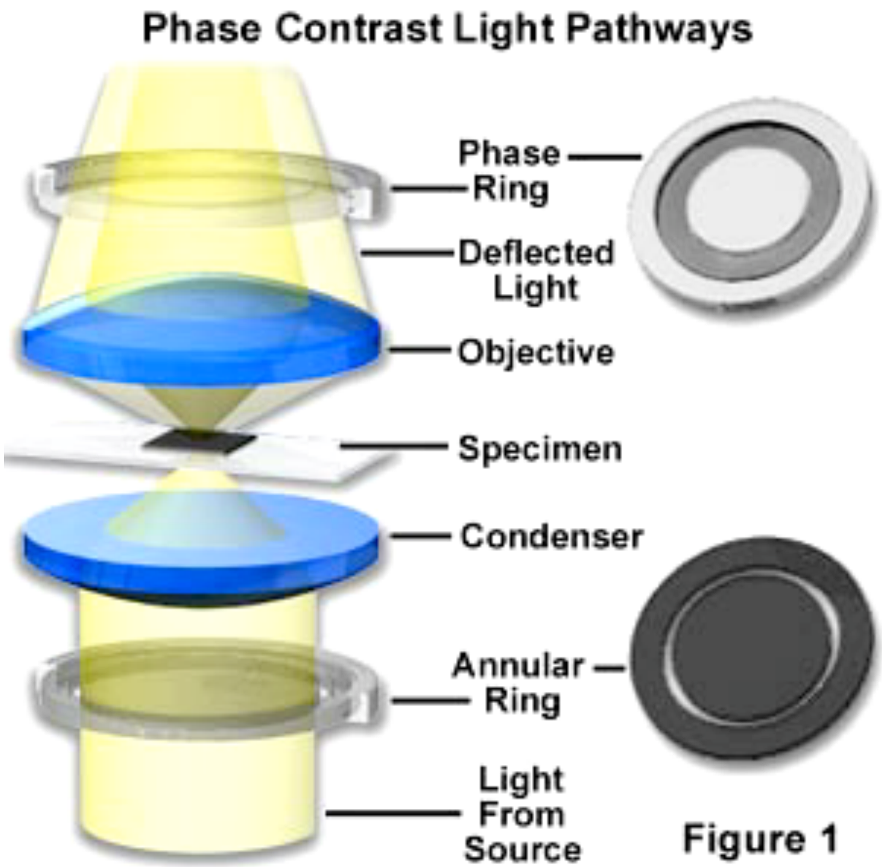
  $$NA = n(\sin \mu)$$

- Because μ cannot be greater than 90° (sin μ = 1), the maximum possible numerical aperture is determined by the refractive index (n) of the immersion medium, including water (n = 1.33), glycerin (n = 1.47), immersion oils (n = 1.52)
- d_{th} = 0.25 μm (λ = 550 nm; NA = 1.40)

Other light microscopic techniques

- **Differential interference contrast (DIC)**
  - a mode of contrast generation in microscopy that uses polarized light and an “analyzing filter”, yielding an image with a shadow relief
- **Dark field**
  - illuminates the specimen but does not admit light directly to the objective
- **Stereo dissection**
  - perceived depth by transmitting twin images that are inclined by a small angle to yield a true stereoscopic effect

Phase contrast microscope



- Phase of the light diffracted by the specimen is altered by approximately 1/4 wavelength
- Human eye is sensitive only to color (light frequency) or to light intensity (wave amplitude)
- Rings cause (destructive or constructive) interference - better contrast
- Zernike received Nobel Prize in 1953

Fluorescence microscope

- Excitation light irradiates the specimen
- Fluorescent light is separated from the brighter excitation light and only the emission light reaches the eye
- Fluorochromes are specific in their attachment to biological structures
- Energy-transfer fluorochromes

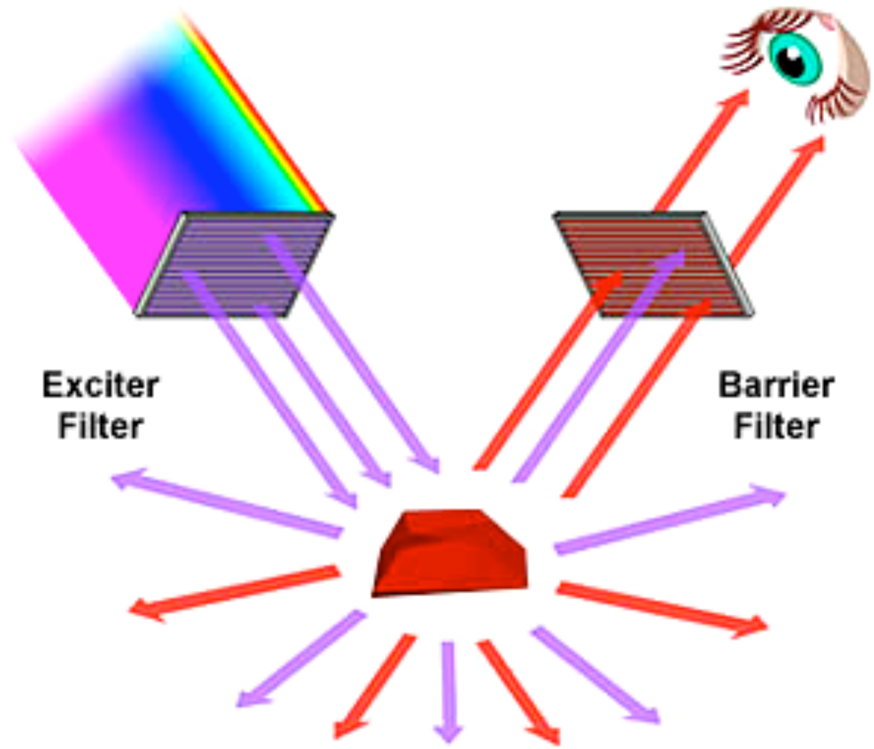
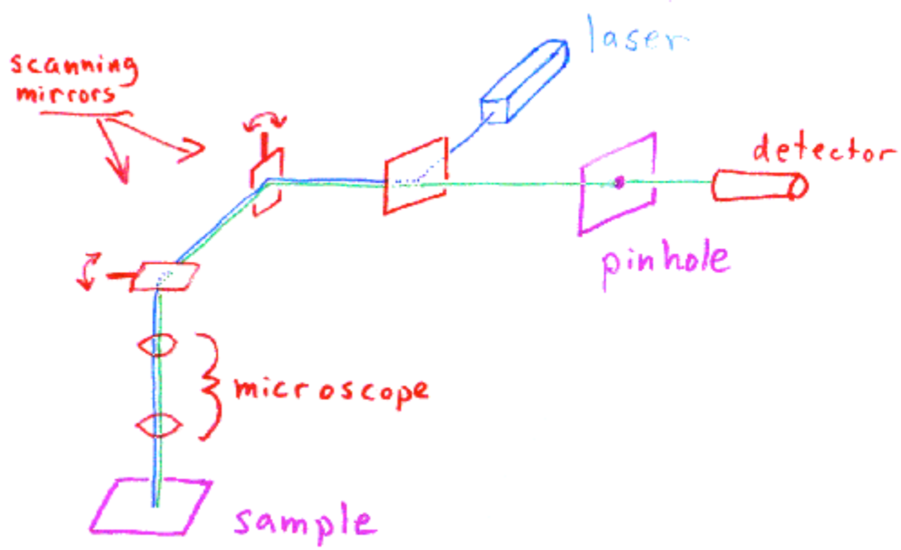


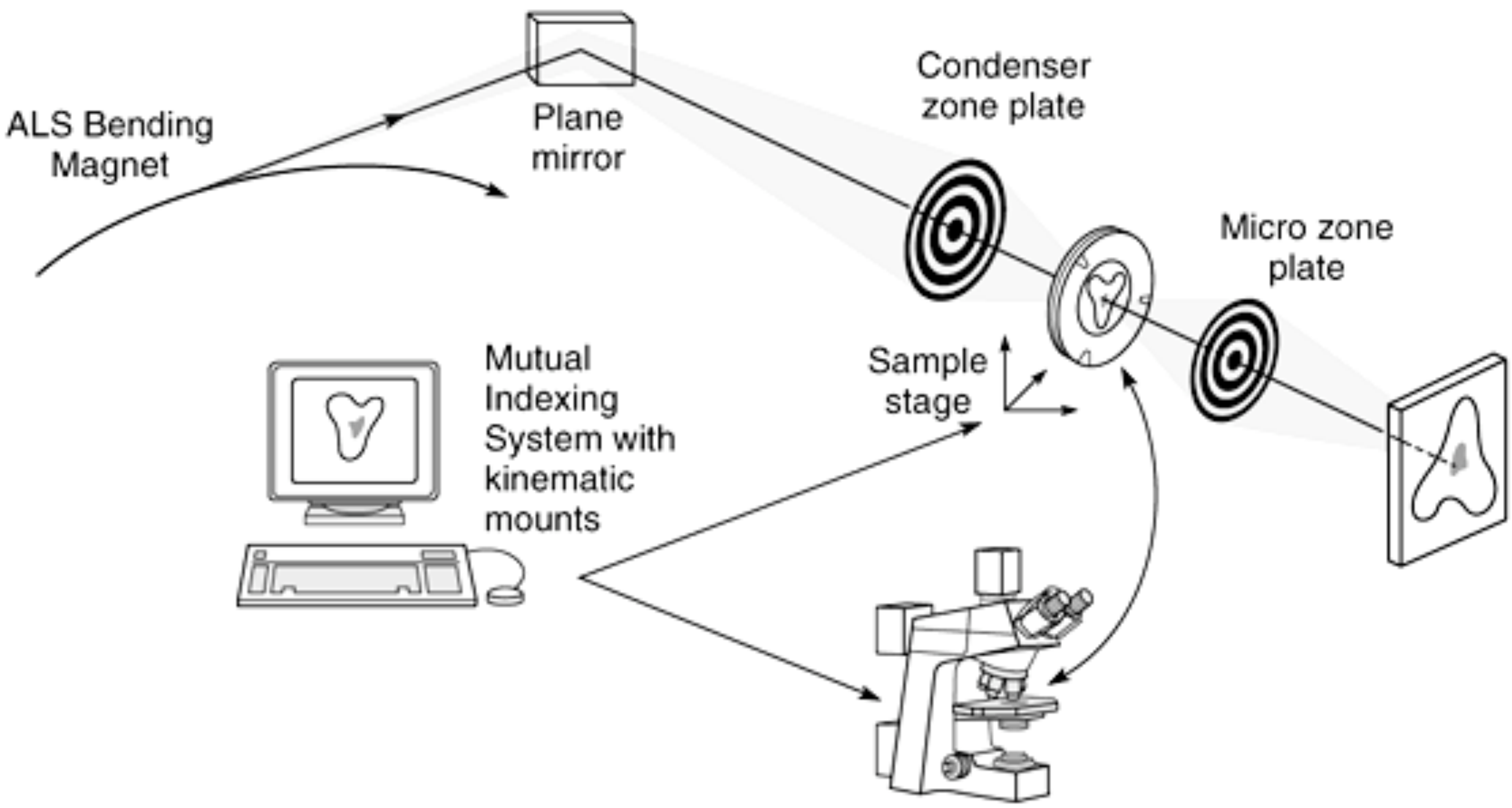
Figure 3

Laser scanning confocal microscope

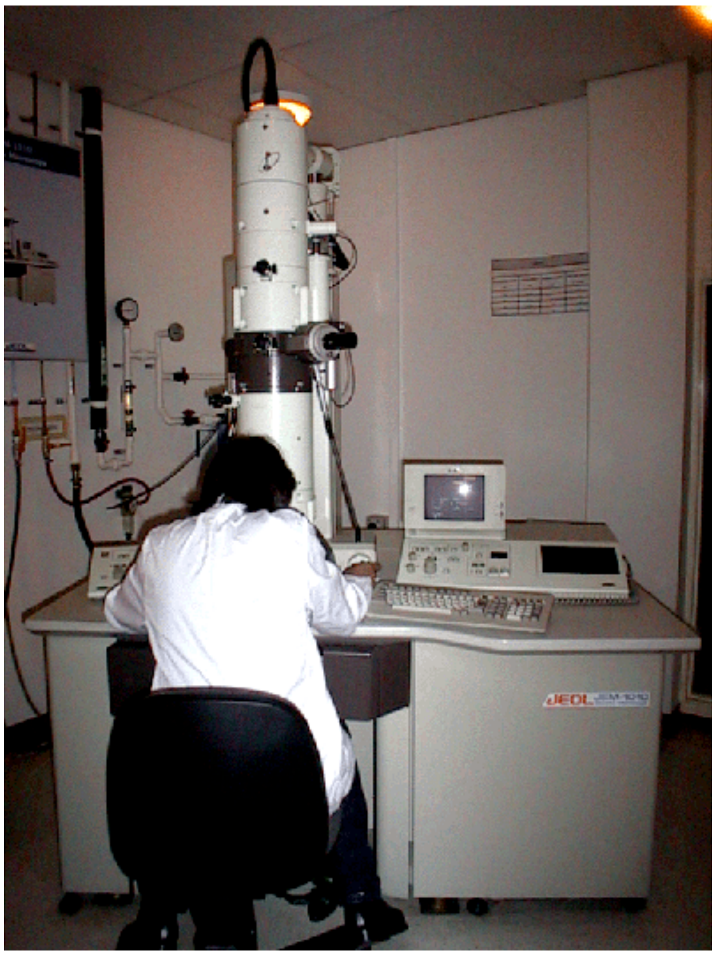


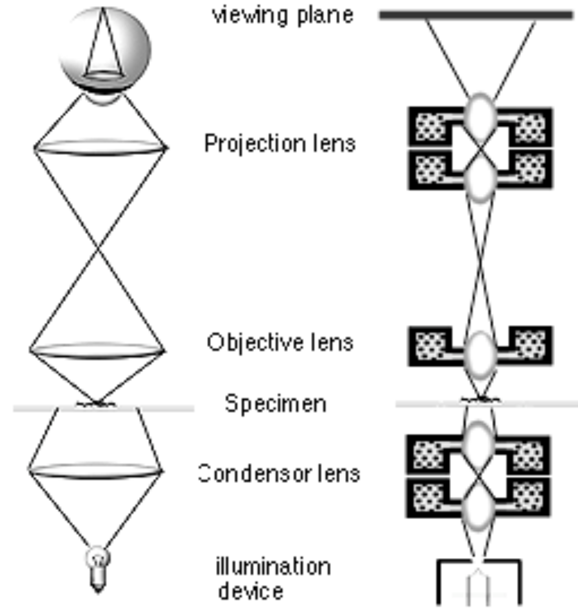
- Resolution: 0.2 µm
- Optical sectioning results in 3D image
- Limited by scanning speed

X-ray microscope



Transmission electron microscope

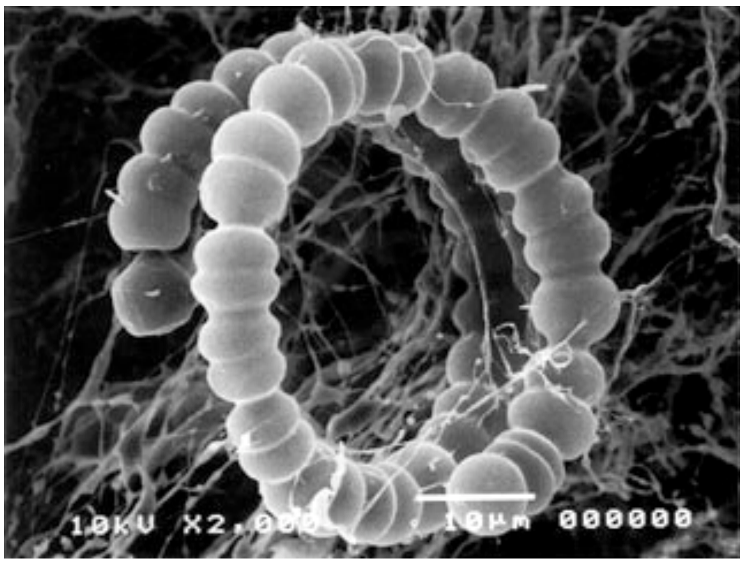


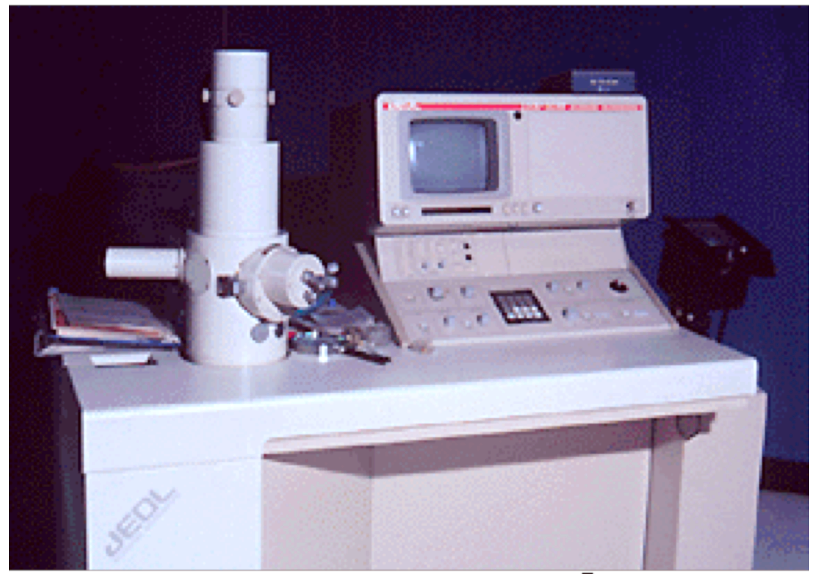


- Electron wave (λ < 0.005 nm)
- Thousand fold increase in resolution, hundred fold in depth of field
- High vacuum needed
- Extensive sample preparation
- Sample is always dead, images often contain artifacts

Scanning electron microscope

- Magnification ranges from 15x to 200,000x
- Resolution: 5 nm





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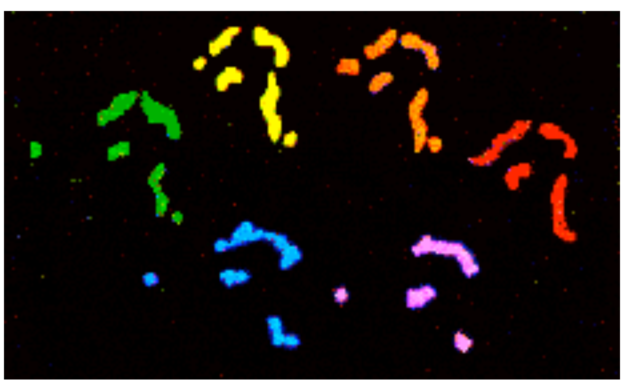
Atomic force microscopy



- Image at atomic resolution
- In the non-contact mode (distance >10Å between the tip and the sample surface), Van der Waals, electrostatic, magnetic or capillary forces produce images of topography
- In the contact mode, ionic repulsion forces take the leading role

A Window on the Future

Consider gallium nitride, a semiconductor whose promising applications include light-emitting diodes that produce a rainbow of colors: until recently it was impossible to make atomic-resolution images of such a material. Under a transmission electron microscope, heavy atoms like gallium scatter electrons so much that they swamp the signal from lightweight neighbors like nitrogen. Then the One-Angstrom Microscope (OÅM) debuted at Berkeley Lab's National Center for Electron Microscopy (NCEM).

Modern Microscope Component Configuration